REMARKS

Claims 1-18 are pending in the application. Claim 1 has been amended herein to recite that the embryonic stem cells have the ability to differentiate into three primary germ layers and also germ cells, support for which may be found in the present specification. Applicants respectfully submit that no new matter has been added by way of the present claim amendment.

Rejection Under 35 U.S.C. 112, First Paragraph

Claims 1-18 stand rejected as containing new matter. Applicants respectfully submit that the previous claim amendment was the result of an inadvertent typographical error. However, the present claims have been properly amended in accordance with the present invention.

Applicants provide the following comments regarding the present claim amendment. Moreover, Applicants note that in the last Office Action, the Examiner did not acknowledge any of the scientific periodicals which were used to establish what those of ordinary skill at the time of the present invention would understand regarding the ability of the recited embryonic stem cells to differentiate into three primary germ layers and also germ cells. Applicants respectfully request that the Examiner consider the articles, which are again submitted for the Examiner's consideration, and provide comments regarding the articles, in the event that the Examiner does not find them to support the present claim amendment.

Support for the Present Claim Amendment

Applicants acknowledge that literal support for the present claim amendment is not present in the specification. However, the subject matter of the claim need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement. The test for sufficiency of support in a parent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting *In re Kaslow*, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)).

Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. See, e.g., *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998); *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997); *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it").

Here, it is clear from a reading of the specification that the present inventors had possession of the presently claimed invention. Moreover, the scientific periodicals at the time of the present invention also indicate that those of ordinary skill in the art would appreciate that the present inventors had possession of the presently claimed invention. Applicants submit herewith evidence (i.e., scientific periodicals) that those of ordinary skill in the art at the time of the present invention would understand that "embryonic stem cells" (as used by the inventors in this application) would "have the ability to differentiate into three primary germ layers and also germ cells". Applicants offer a brief statement of the relevance of each of the evidentiary documents below, for the Examiner's convenience.

Thomson et al., "*Isolation of primate embryonic stem cells*", PNAS, Vol. 92, pp. 7844-7848, (1995).

This article relates to established embryonic stem cells of a monkey. In particular, at the left column on page 7844, it is disclosed that "Embryonic stem (ES) cells, derived from preimplantation embryos (1,2) and embryonic germ (EG) cells derived from fetal germ cells (3,4), are undifferentiated immortal cells capable of differentiating into derivatives of all three embryonic germ layers."

Thomson et al., "*Embryonic Stem Cell Lines Derived from Human Blastocysts*", Science, Vol. 282, pp. 1145-1147, (1998).

This article relates to established human embryonic stem cells. The summary discloses that "Human blastocyst-derived, pluripotent cell lines are described that have Norman karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm), cartilage bone, smooth muscle and striated muscle (mesoderm); and neural epithelium, embryonic ganglia and stratified squamous epithelium (ectoderm)."

Also, in the right column on page 1145, it is disclosed that "The human ES cell lines surface markers that characterize undifferentiated nonhuman primate ES and human EC cells, including stage-specific embryonic antigen (SSEA)-3, SSEA-4, TRA-1-160, TRA-1-81, and alkaline phosphatase." However, the Examiner should note that these markers are specific to human embryonic stem cells, so as not to be expressed in the cells disclosed in Weiss.

Therefore, in light of the apparent appreciation of the scope of the present disclosure by those of ordinary skill in the art, Applicants respectfully submit that the present claim amendments are fully supported and do not constitute the addition of new matter to the specification.

It is also submitted that the present claim amendments overcome the previous prior art rejections. The distinctions between the present invention and the cited prior art are reiterated below for the Examiner's convenience.

Rejections under 35 USC § 102

Claims 1-8 and 10-18 were previously rejected under 35 U.S.C. § 102(b) as being anticipated by US Patent 5,981,165 to Weiss et al. (hereinafter "Weiss"). Applicants respectfully traverse.

Applicants respectfully maintain that the Examiner appears to misunderstand Weiss. In Weiss, although the title at column 12, Example 3 recites "Isolation and Propagation of Embryonic Stem Cells", it is disclosed at lines 24-25 that "neurospheres" are actually formed. Thus, the title should have been "Isolation and Propagation of Neural Stem Cells". The embryonic stem cells used by Weiss are different from those presently claimed. The embryonic stem cells in Weiss actually mean stem cells (i.e., neural stem cells) prepared from embryonic tissue (or embryonic brain). That is, Weiss uses "embryonic stem cells" as a common term to indicate neural stem cells from an embryo. This is apparently a misuse in comparison with the current definition. *See* both Thomson articles discussed above.

As Applicants have previously argued, embryonic stem cells have an ability of differentiating into all of the cells – ectoderm, endoderm, or mesoderm. Present claim 1 has been amended to affirmatively recite this feature of the present invention. However, there has not been an instance where neural stem cells prepared from the brain are differentiated into germ cells. Accordingly, the presently claimed embryonic stem cells are quite different from the neural stem cells of Weiss. Thus, the present invention cannot be anticipated from Weiss.

Rejections under 35 U.S.C. § 103

Claims 1-3 and 10-12 were previously rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. (hereinafter "Zhang") in view of Flax et al. (hereinafter "Flax"). Applicants respectfully traverse.

Zhang reports that human embryonic stem (ES) cells cannot be maintained in an undifferentiated state in the absence of FGF-2 (Ref. 4 of Zhang). From this report, it is

considered in Zhang that the differentiation occurs by removing FGF-2 and forming embryonic bodies (EB). Therefore, if FGF-2 is added, the differentiation would have been suppressed.

In the neural stem sphere used in the present invention, the suppression of differentiation into the neural stem cells (NSC) does not take place even when FGF-2 is added upon suspension culture in ACM. Thus, the present invention possesses an unexpected synergistic effect, contrary to that described in Zhang, which enhances the differentiation. The effect for FGF-2 upon the suspension culture as described above is reversed; therefore, it is clear that the present invention is completely different from that taught in Zhang.

The method of Zhang allows differentiation of the cells by an EB method to form neural tube-like structure on day 7 of the adhesion culture. By contrast, in the present invention, a sufficient amount of neural stem cells (NSC) are differentiated day 4 after the suspension, more in the suspension culture, not the adhesion culture. In the present invention, it is reasonable to consider that the reason that the NSC are differentiated only on a surface layer of the neural stem sphere is that a factor in ACM penetrates into the neural stem sphere, thereby enhancing the differentiation into NSC, proving that the ES cells are subjected to direct differentiation.

The NSC are reportedly neuroepithelial stem cells and radial glia of the ventricular zone in embryo, or astrocytes of the subventricular zone and subgranular zone in adult. The fact that the NSC are differentiated in the order of neuroepithelial stem cells → radial glia → astrocytes, strongly suggests that the differentiation into astrocytes occurs in a default state of brain development.

Therefore, the fact that almost all of the ES-derived NSC are differentiated into astrocytes by removing FGF-2 agree with the phenomenon in the development of the brain in a living body.

Upon the differentiation into the neural cells in the brain, firstly the differentiation into neurons occurs, and subsequently the differentiation into astrocytes and oligodendrocytes occurs

in accordance with the progress of the time axis. The ES-derived NSC of the present invention are differentiated into astrocytes in a default state as mentioned above, while almost all of the cells are differentiated into neurons by providing an exogenic differentiation stimulation called ACM.

The NSC of Zhang are differentiated into three kinds of neural cells, namely neurons, astrocytes, and oligodendrites. Taking into consideration the axis of time from the development of the brain, it is understood that the neural stem cells of the present invention are more undifferentiated than those of Zhang, so that the cells of the present inventions seem to exhibit the nature close to that of the neuroepithelial stem cells.

In order that small elongated cells congregated in the center shown in Fig. 1-A of Zhang form a neural tube-like structure shown in Fig. 1-B with the time course, Zhang merely mentions an experimental tool to study human neural tube formation under controlled conditions (Zhang, p. 1131, second column, second paragraph), i.e. ES cell-derived neural precursor cells, recapitulate early steps of nervous system development in that neural tube-like structures are formed, merely stating that the process of development is reproduced. Therefore, Zhang does not directly relate to the differentiation of the ES cells into NSC.

Flat cells are migrated in the periphery of the adherent EBs; however, these cells are negative against markers for neurons, astrocytes, oligodendrites, and ES cells. Therefore, under the conditions of Zhang, many of ES cells are differentiated into unidentified cells; therefore, it is obvious that the ES cells cannot be directly differentiated into NSC. An advantage of the Zhang method is to collect the cells only having a rosette structure utilizing the difference in adhesion from unidentified flat cells, but never describing that Zhang performs direct differentiation of the ES cells.

A medium DMEM/F12 plus supplements used in Zhang is a general basic medium for preparing an astrocyte conditioned medium, and the medium is completely different from ACM

containing various factors produced by astrocytes. As mentioned above, the method of Zhang merely prepares EB by removing FGF-2; therefore, Zhange does not render the present invention obvious.

Incidentally, the article contribution of Flax describes NSC collected from human fetal telencephalon that are cryopreserved, so that Flax is distinguishable from the teachings of the present invention in the cell species.

Rejections under 35 USC § 102/103

Claims 1-18 were previously rejected under 35 U.S.C. §102(b) as anticipated by Zhang et al. or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Pataky et al. (hereinafter "Pataky"). Applicants respectfully traverse.

The comments regarding Zhang in the context of the discussion of the above 35 U.S.C. § 103(a) rejection are likewise applicable to the outstanding rejection.

Further, Pataky does not cure the deficiencies discussed with regard to Zhang. Pataky reports that neuronal axons of the CNS are regenerated in the spinal cord injuries (Refs. 65 and 66 of Pataky). While Pataky makes references to these publications, the main theme of the article is to evaluate what sort of factors enhance survival in regenerable bulbospinal neurons against injuries caused by axotomy.

The effect of enhancing survival of ACM is such that astrocyte-conditioned medium also enhances the survival of bulbospinal neurons, supporting the hypothesis that non-neuronal cells are important mediators of trophic effects observed in vitro (page 366, second paragraph, last sentence of Pataky), to expect the enhancement of the survival by nonneuronal cells (including astrocytes) in the periphery of the injured spinal neurons.

The bulbospinal neurons prepared from E8 Embryo retrograde-labeled with DiI have already ended differentiating into neurons (within the developing chick brain stem, neurogenesis is complete prior to E5; page 367, first paragraph, first sentence of Pataky), so that outgrowth of neurites from bulbospinal neurons is caused by regeneration.

Therefore, Pataky which acknowledges that the differentiation into neurons is ended can no way expect the effect of nerve cell differentiation in ACM.

Accordingly, as described above, the present invention is not obvious over Pataky.

In view of the foregoing, Applicants believe the pending application is in condition for allowance. A Notice of Allowance is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Monique T. Cole, Reg. No. 60,154 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

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Attachments: **Thomson et al., "*Isolation of primate embryonic stem cells*", PNAS, Vol. 92, pp. 7844-7848, (1995).**

Thomson et al., "*Embryonic Stem Cell Lines Derived from Human Blastocysts*", Science, Vol. 282, pp. 1145-1147, (1998).